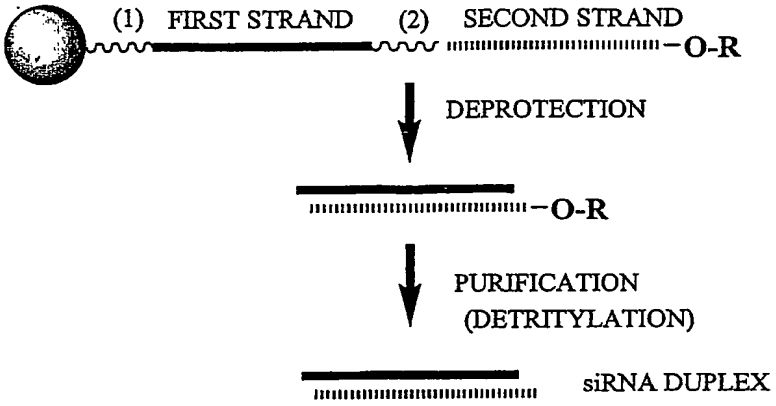
Figure 1

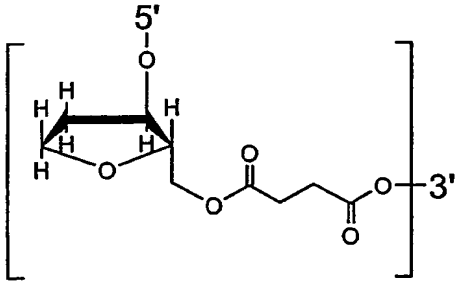


= SOLID SUPPORT

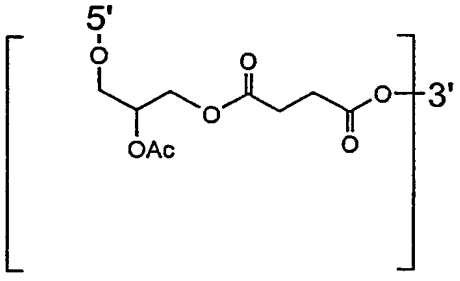
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

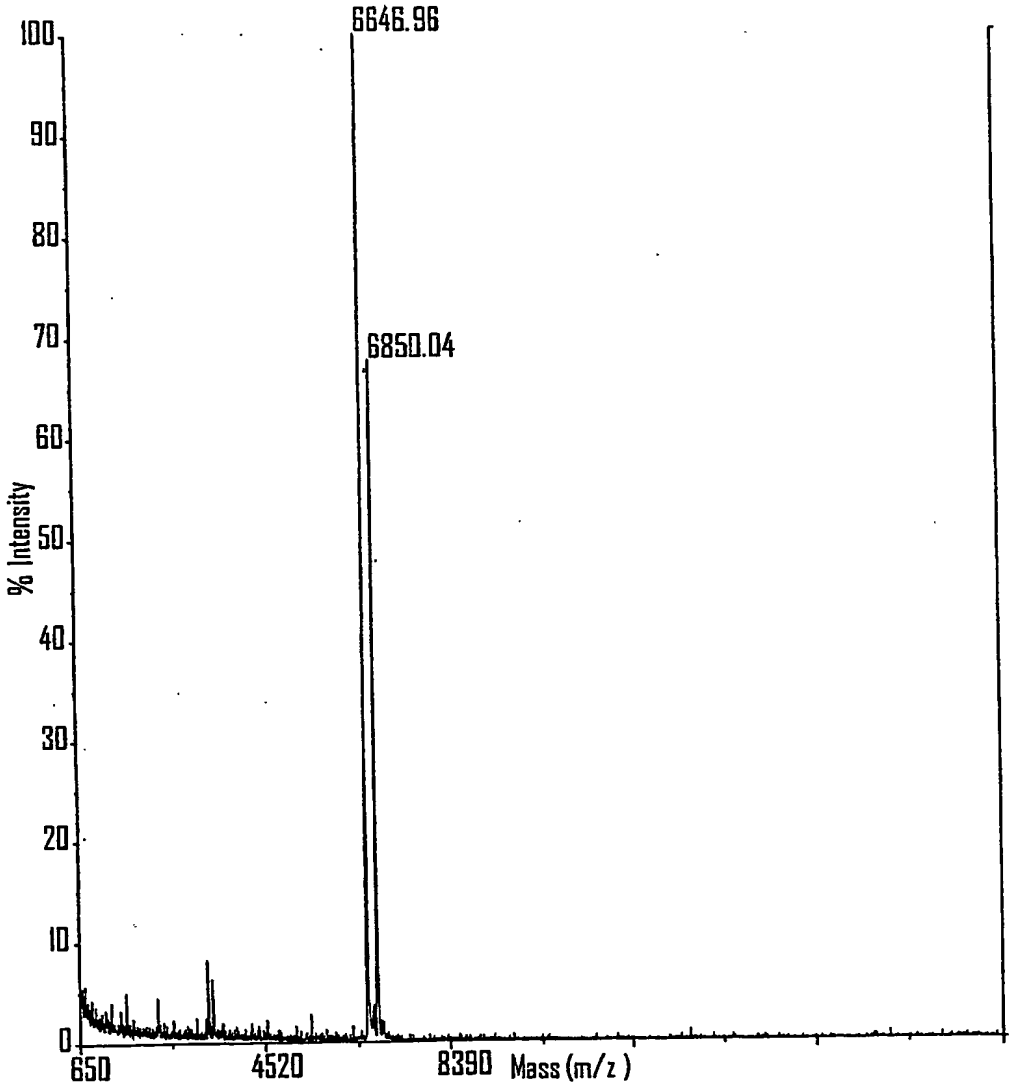

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

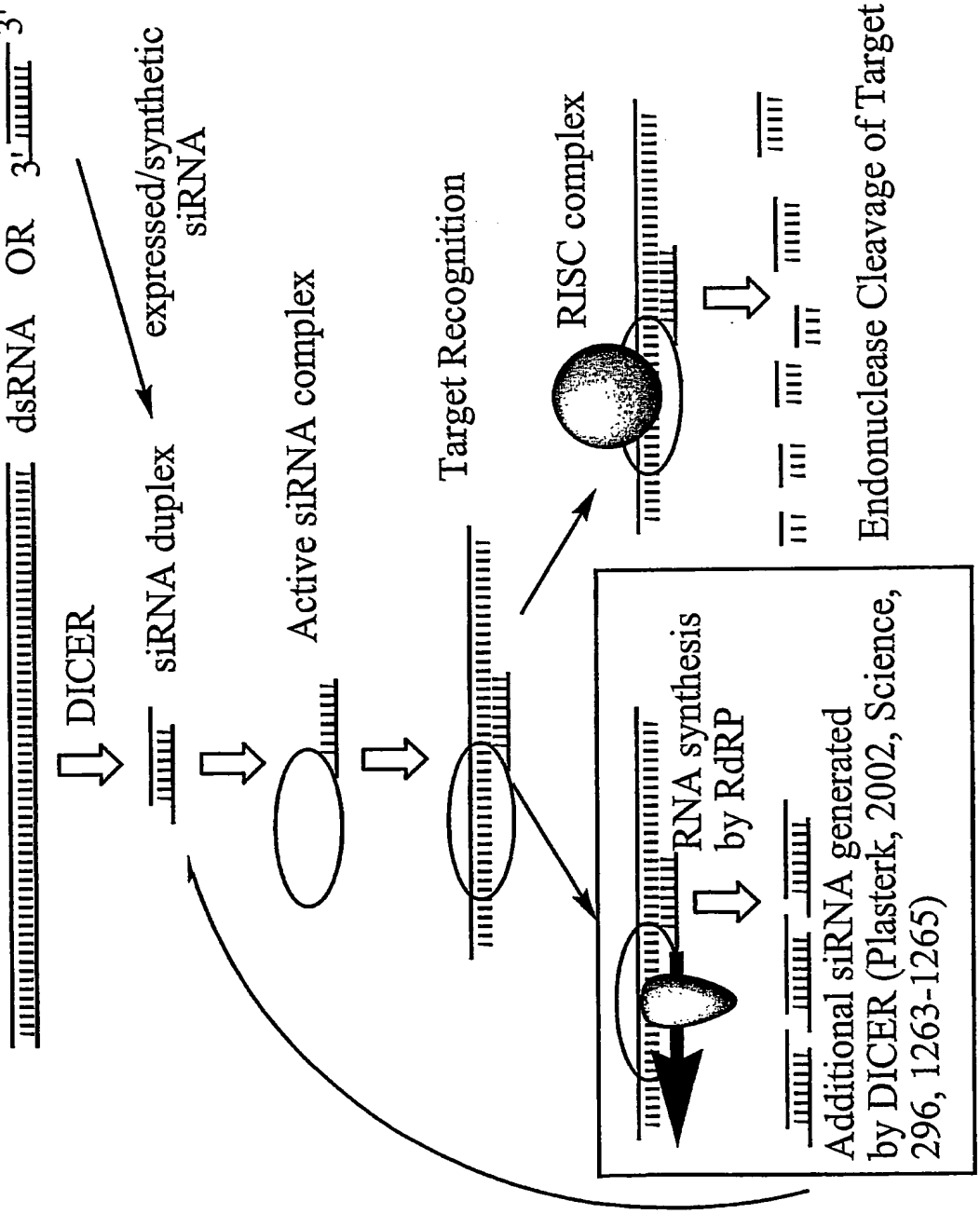
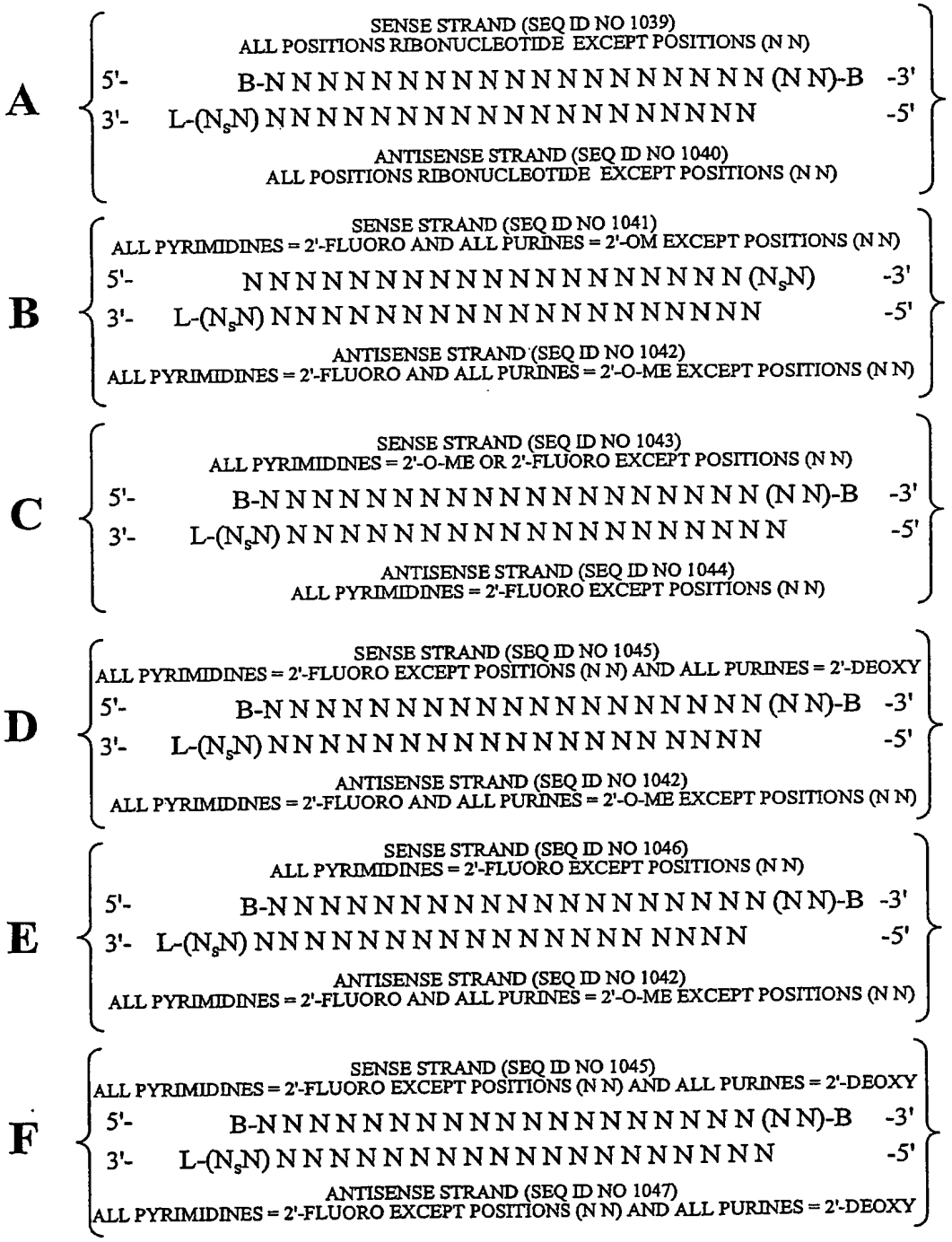
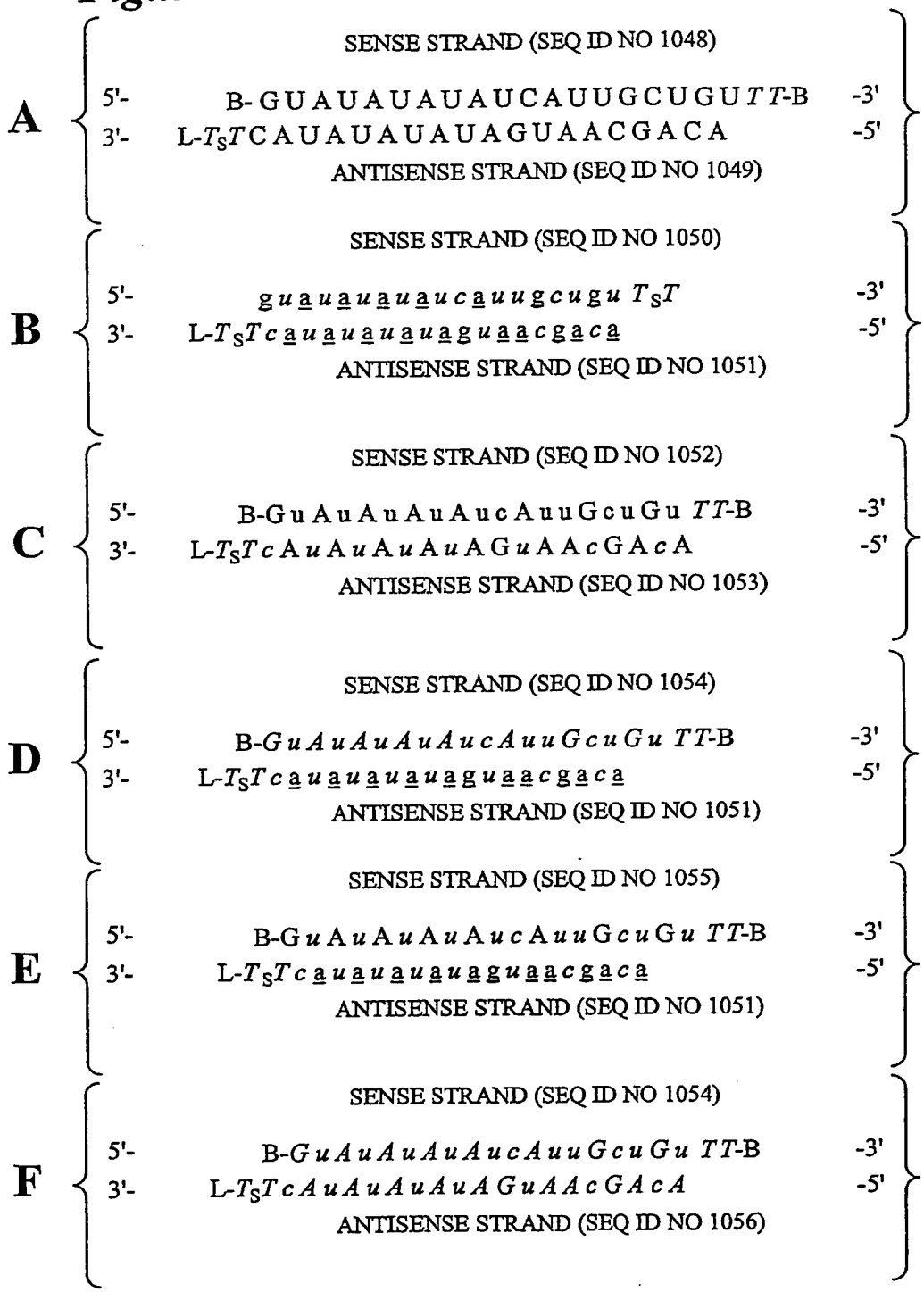

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)
Endonuclease Cleavage of Target

Figure 4



POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL MOIETY THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE THAT IS OPTIONALLY ABSENT

Figure 5



lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY

B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT

L = GLYCERYL MOIETY OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE THAT IS OPTIONALLY ABSENT

Figure 6

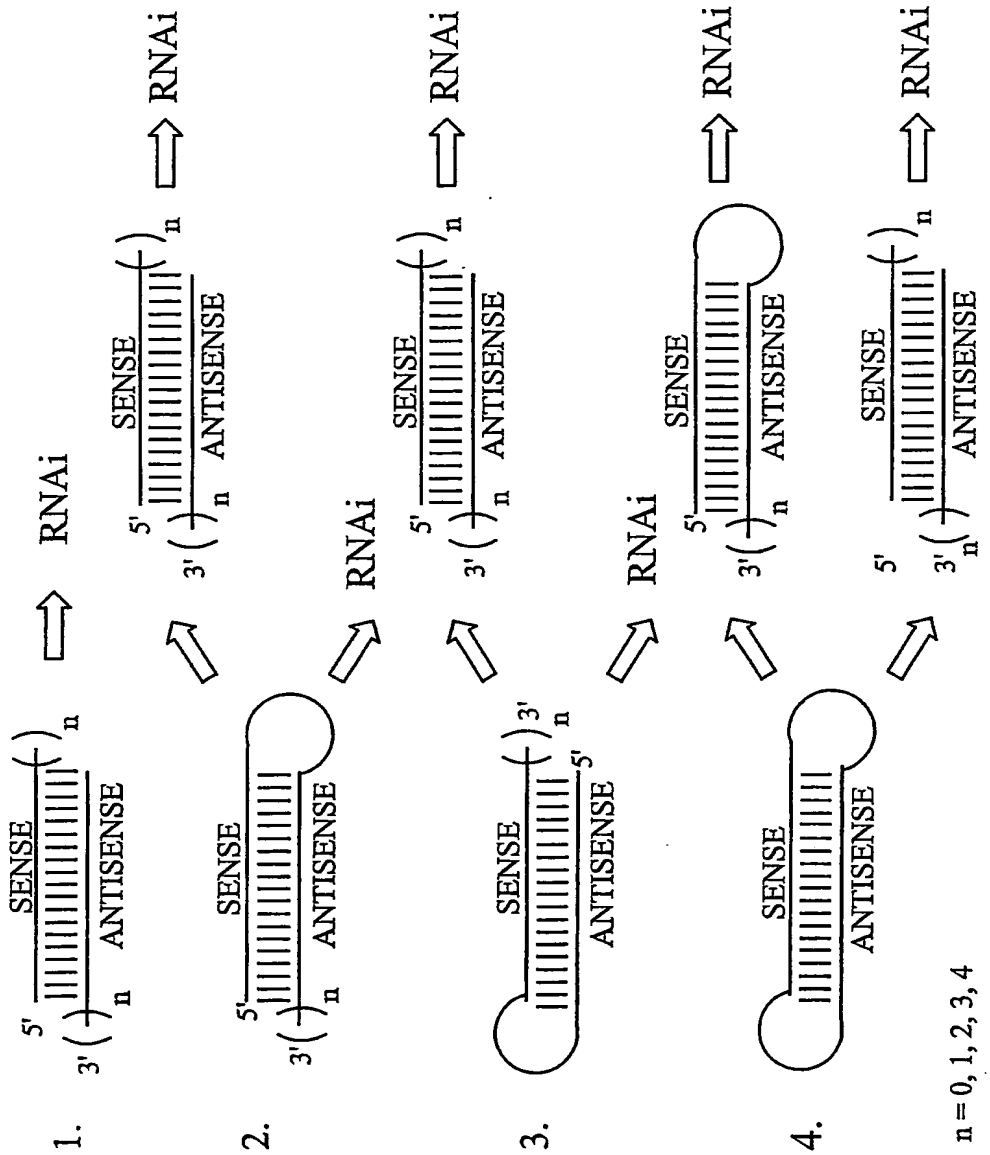


Figure 7

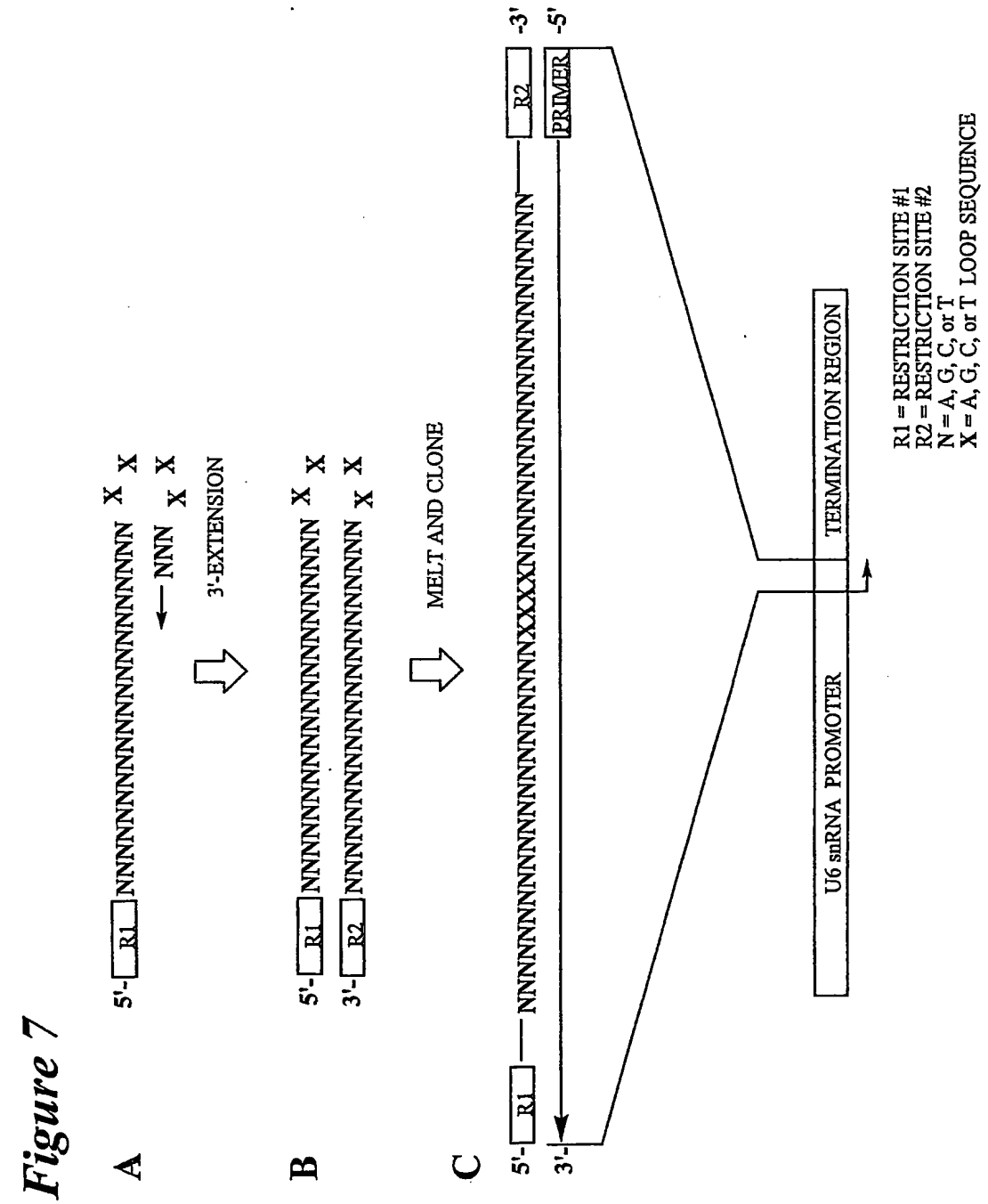
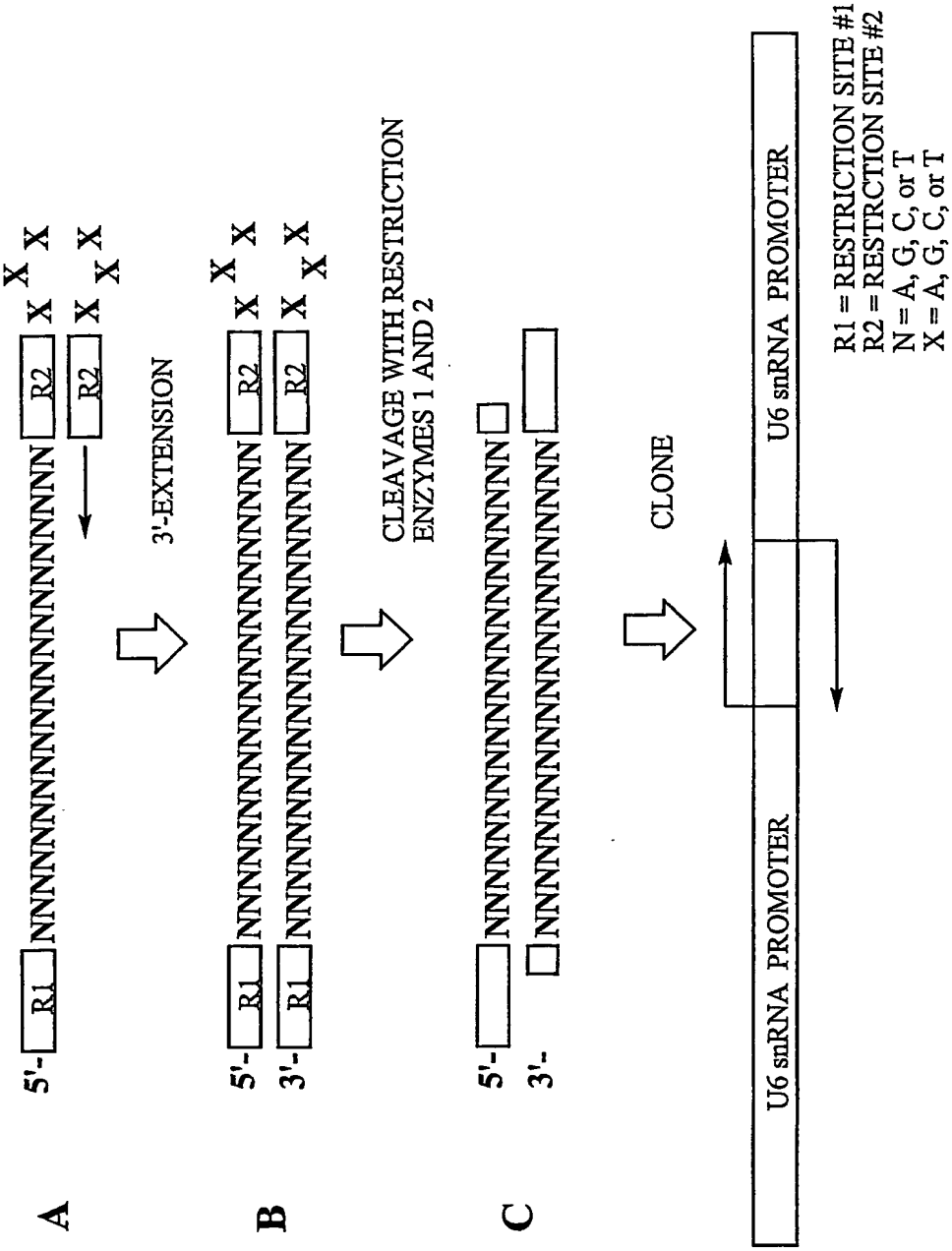

A
5'- R1 NNNNNNNNNNNNNNNNNNN X X
NNN X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 NNNNNNNNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNNN R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8


A
5'- R1 NNNNNNNNNNNNNNNNNNNN R2 X X
R2 X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNNN R2 X X
3'- R1 NNNNNNNNNNNNNNNNNNNN R2 X X
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'- NNNNNNNNNNNNNNNNNNNN
3'- NNNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

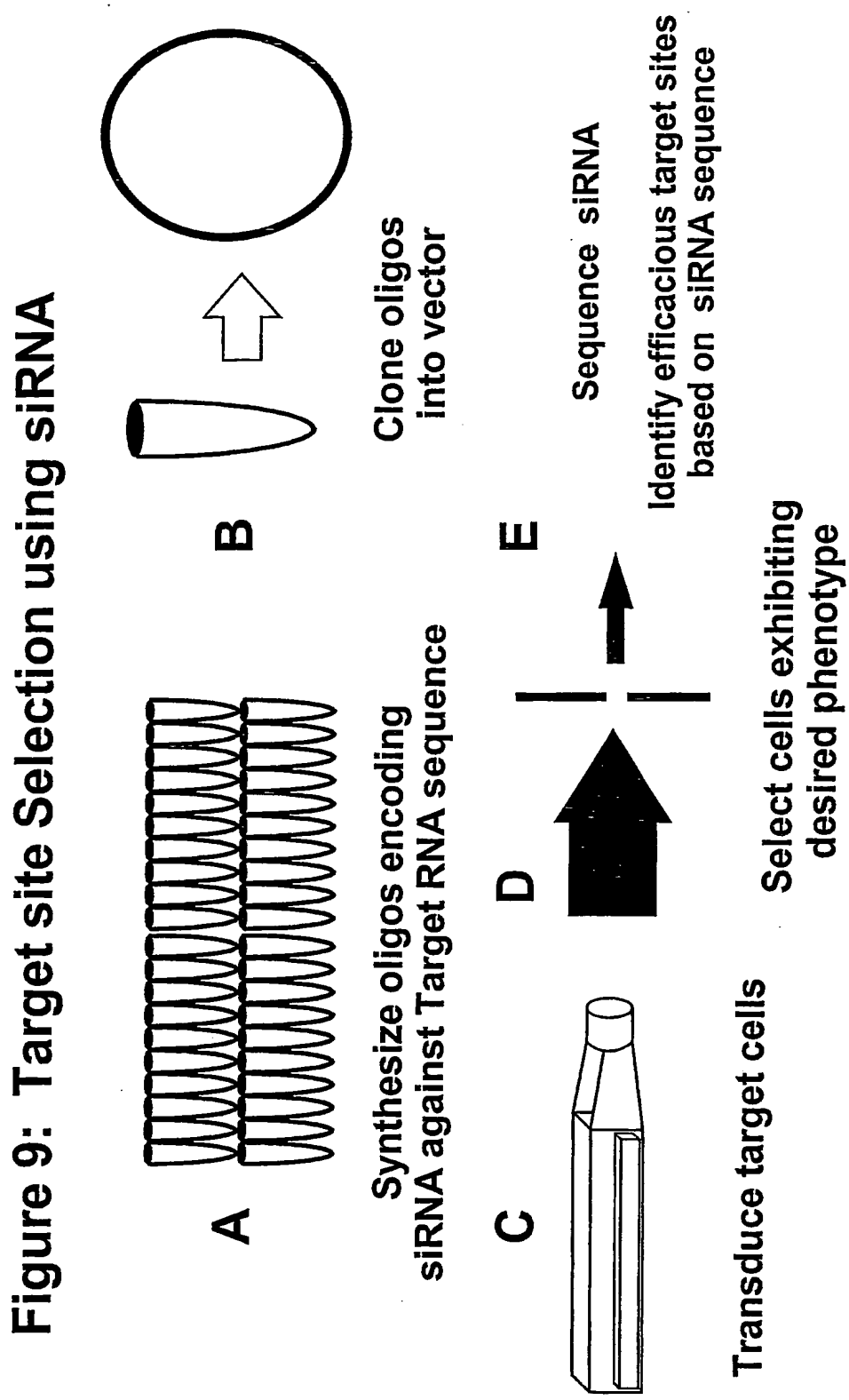
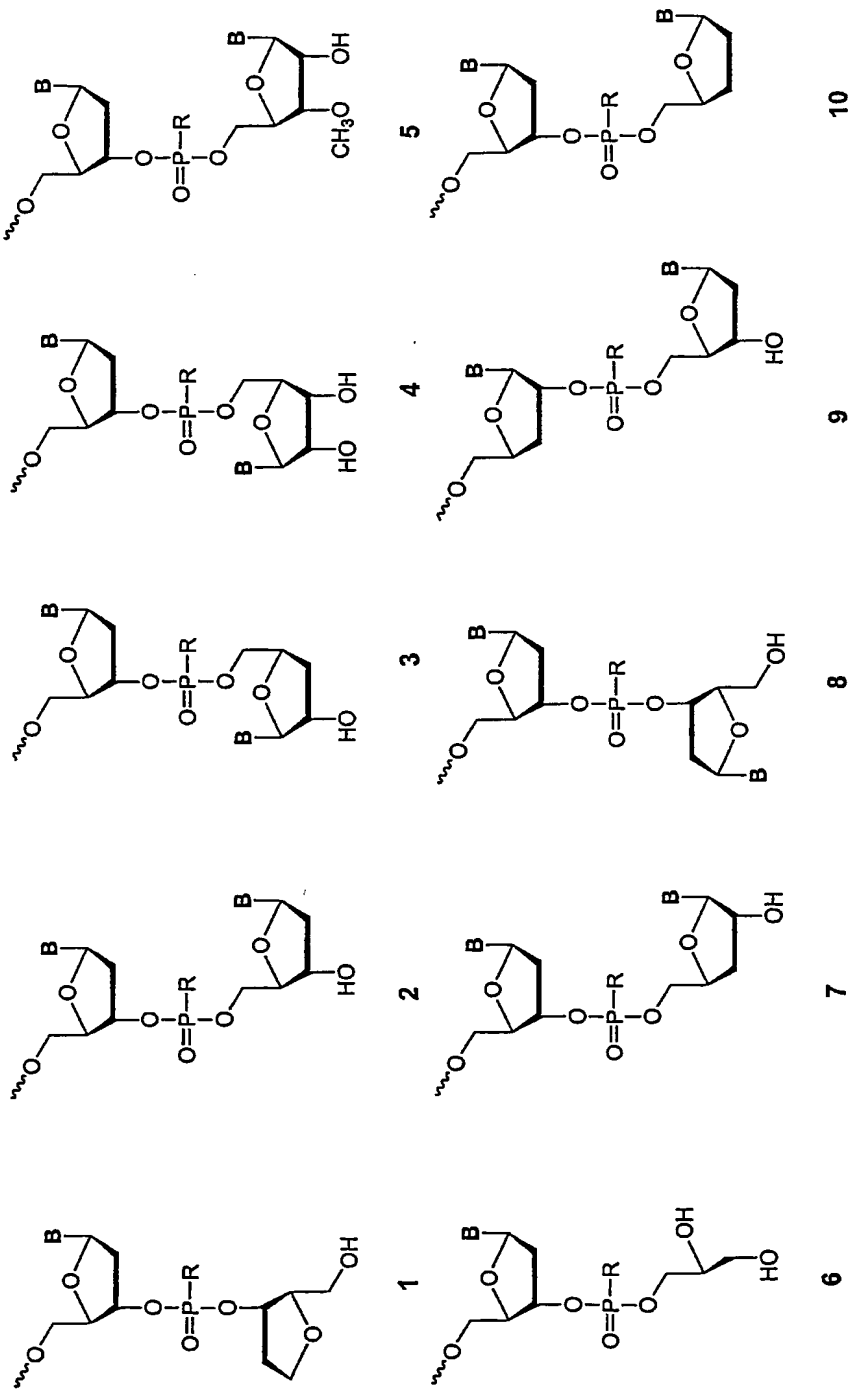


Figure 9: Target site Selection using siRNA
A
Synthesize oligos encoding siRNA against Target RNA sequence
B
Clone oligos into vector
C
Transduce target cells
D
Select cells exhibiting desired phenotype
E
Sequence siRNA
Identify efficacious target sites based on siRNA sequence

Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

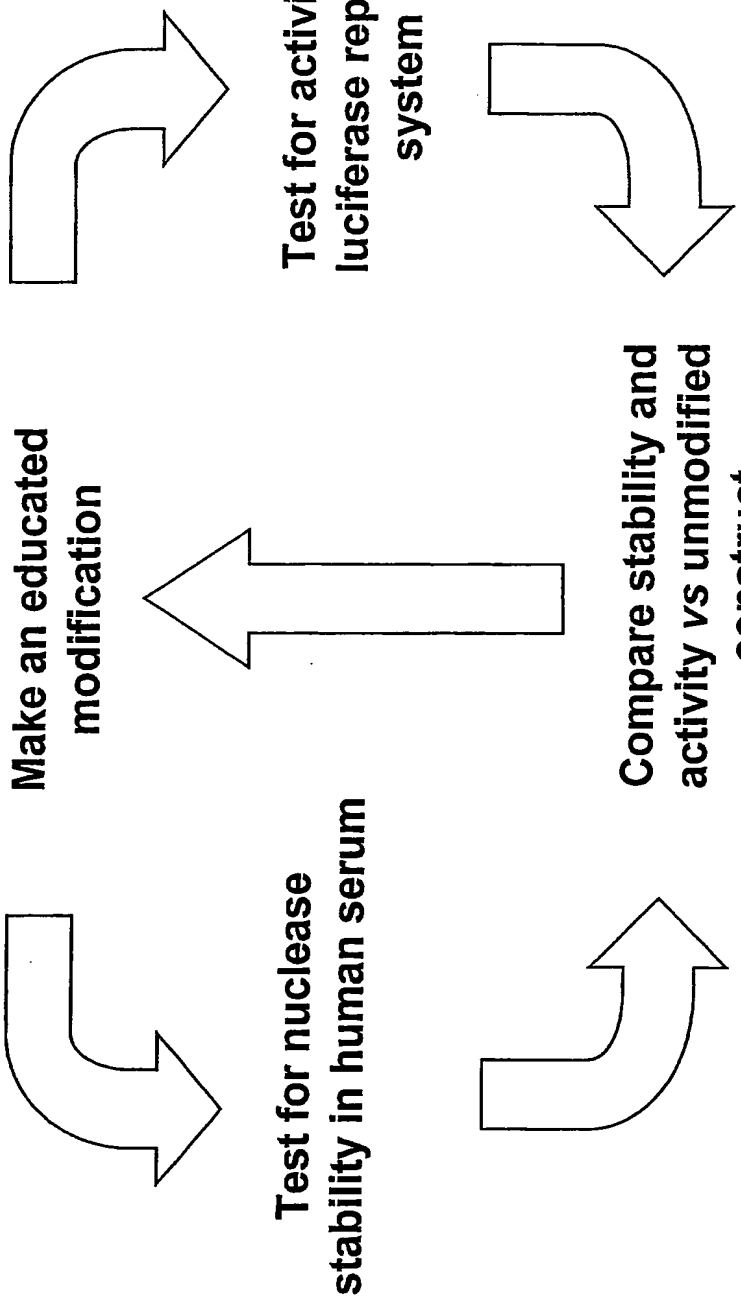
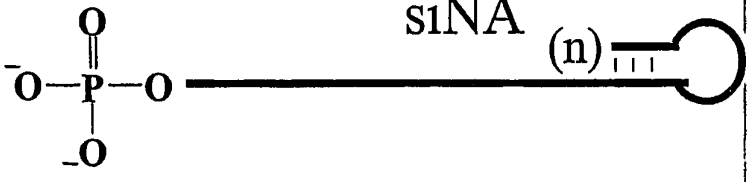


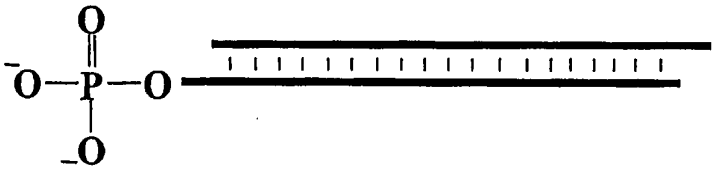
Figure 12: Phosphorylated siNA constructs

Phosphates can be modified as described herein

Asymmetric hairpin siNA



Asymmetric duplex siNA



(n) = number of base pairs (e.g. 3-18 bp)

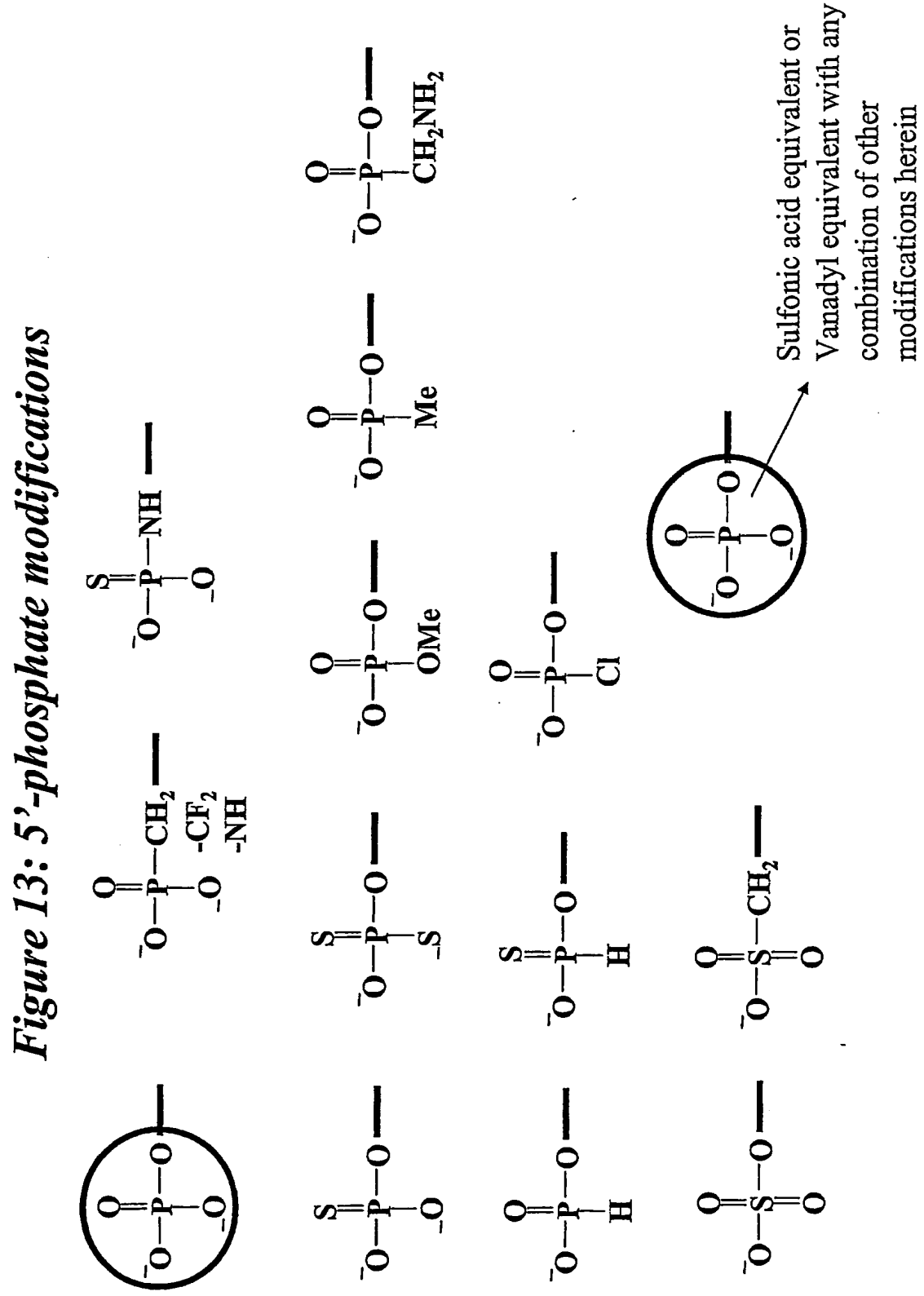
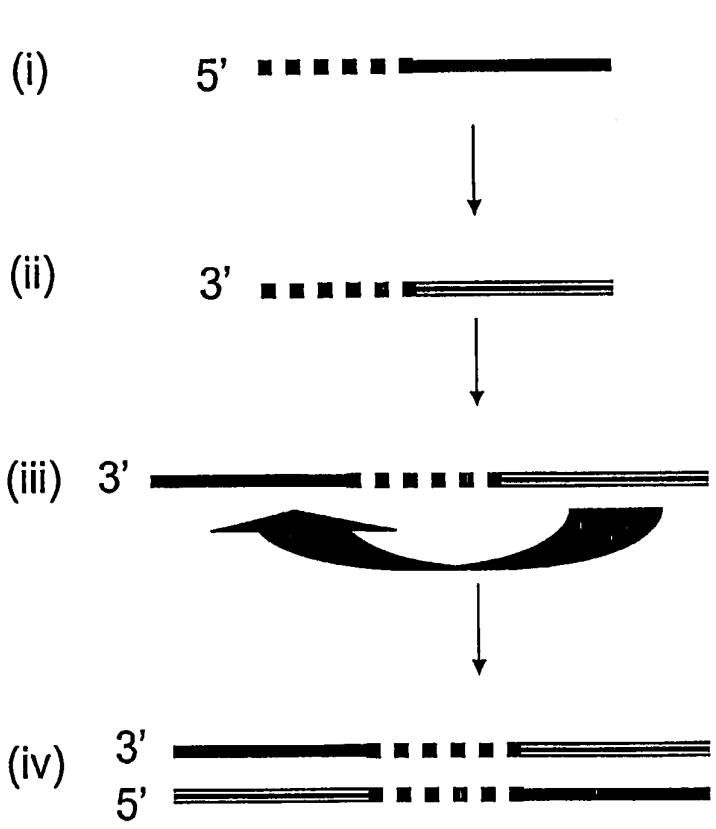


Figure 13: 5'-phosphate modifications
-CF2 -NH
-NH
-OMe
-Me
-CH2NH2
-Cl
-H
-H
-CH2
Sulfonic acid equivalent or Vanadyl equivalent with any combination of other modifications herein

Figure 14A: Duplex forming oligonucleotide constructs that utilize Palindrome or repeat sequences



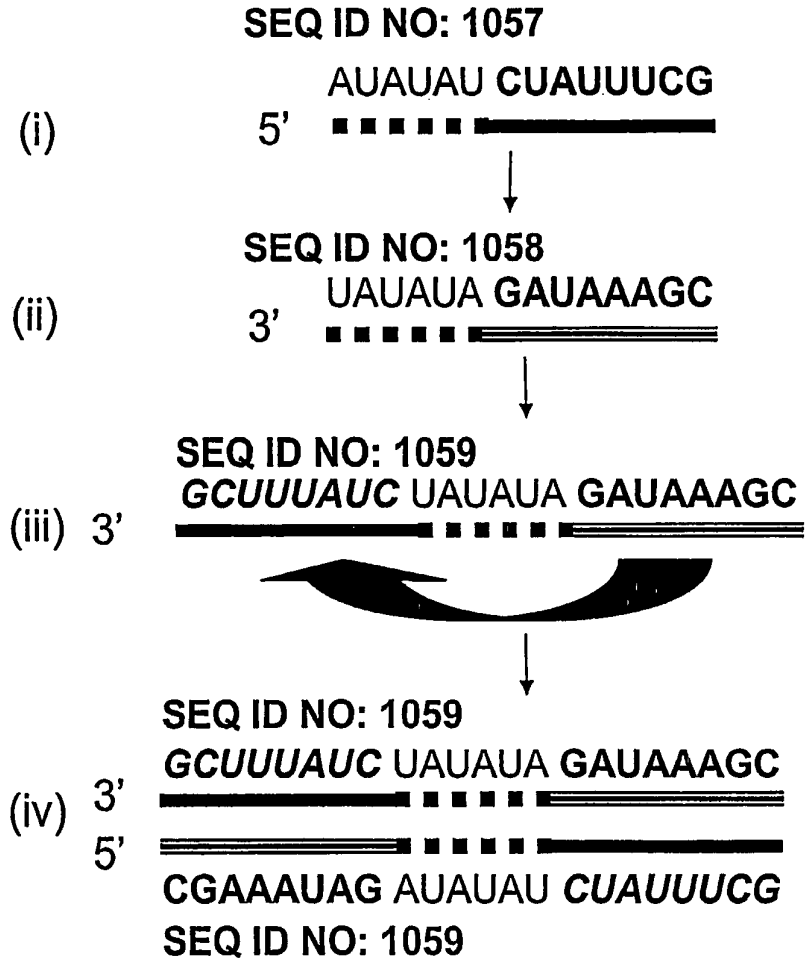
Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct

Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct (blunt ends)

Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

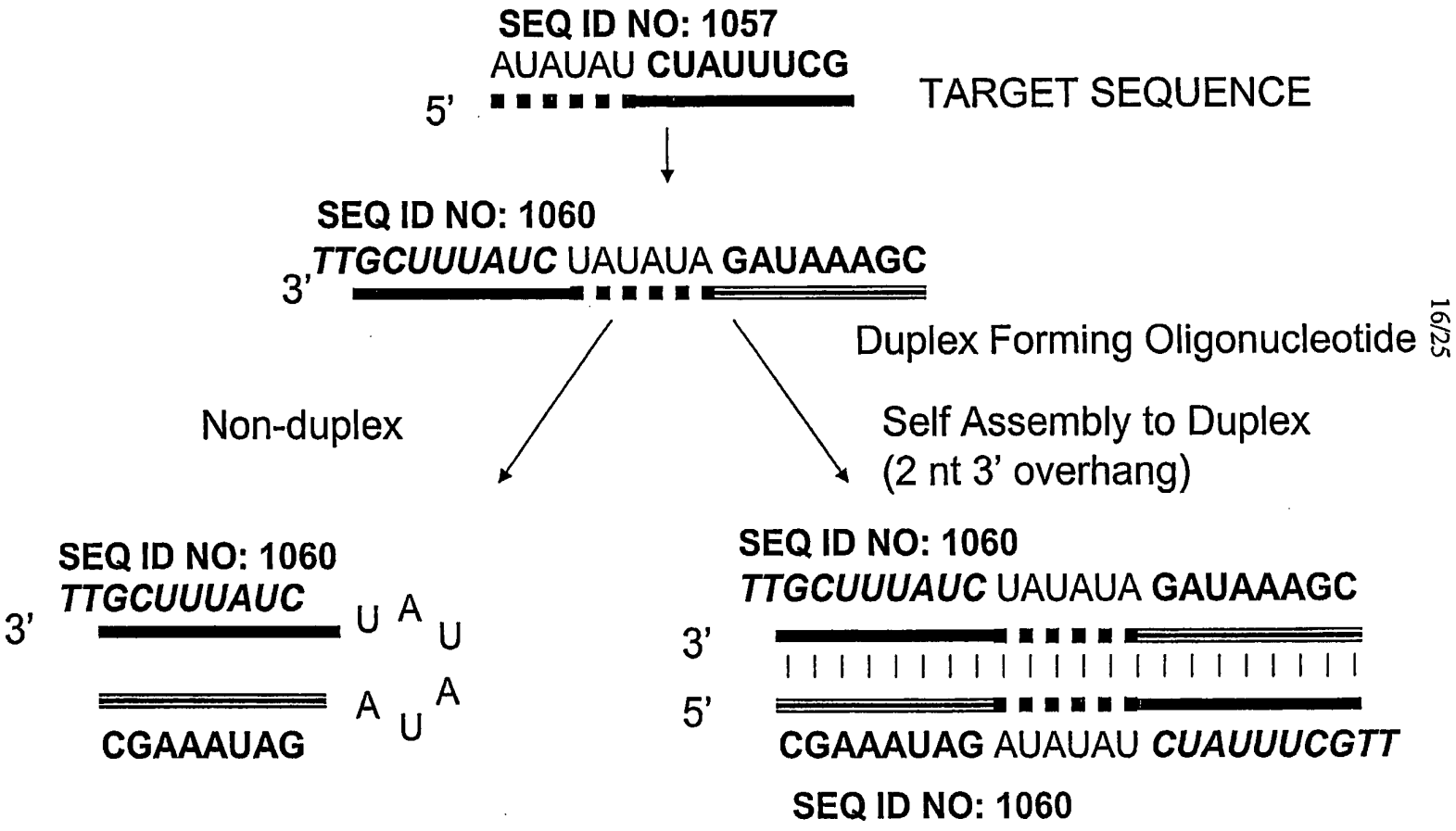


Figure 14D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression

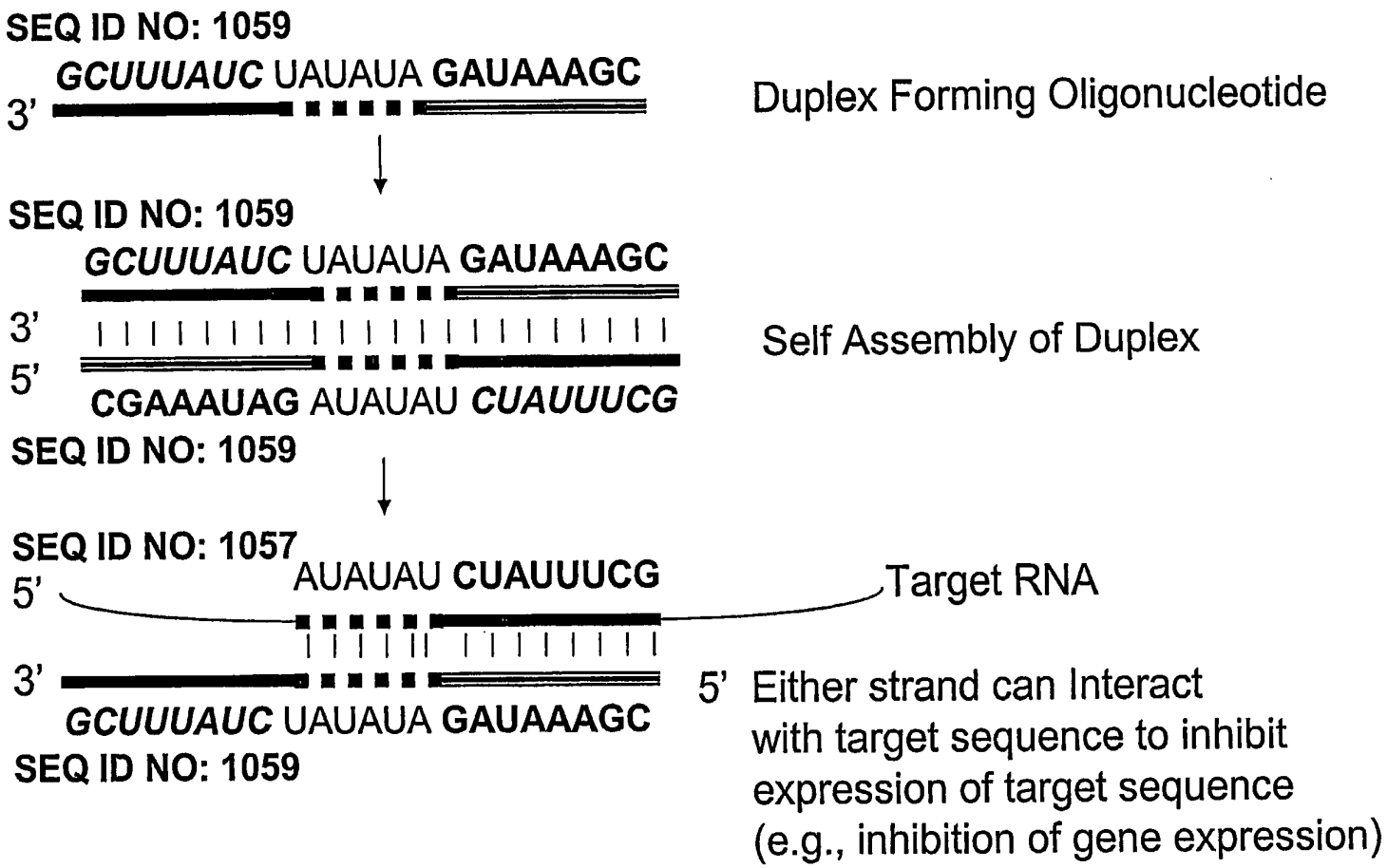


Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences

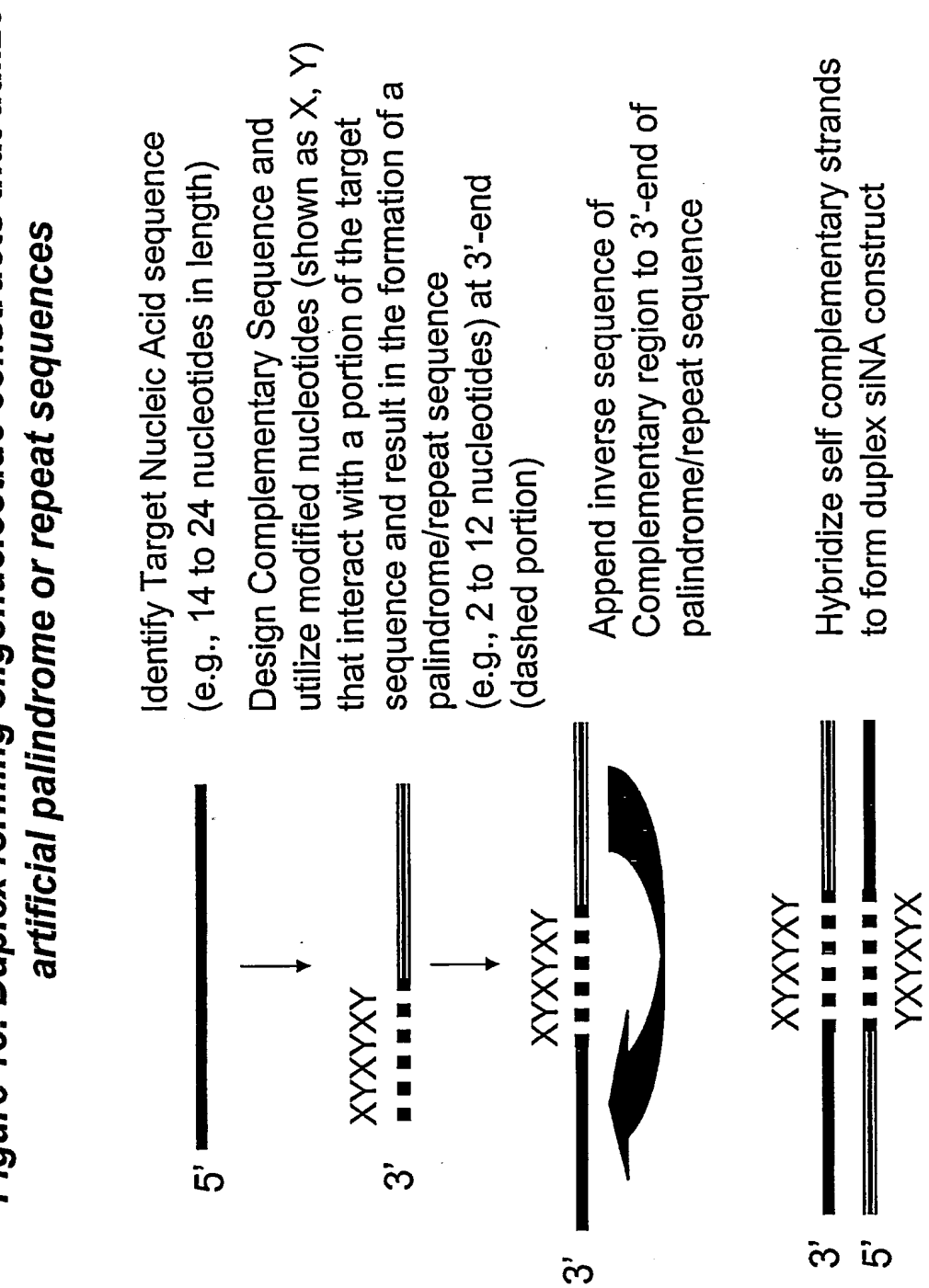
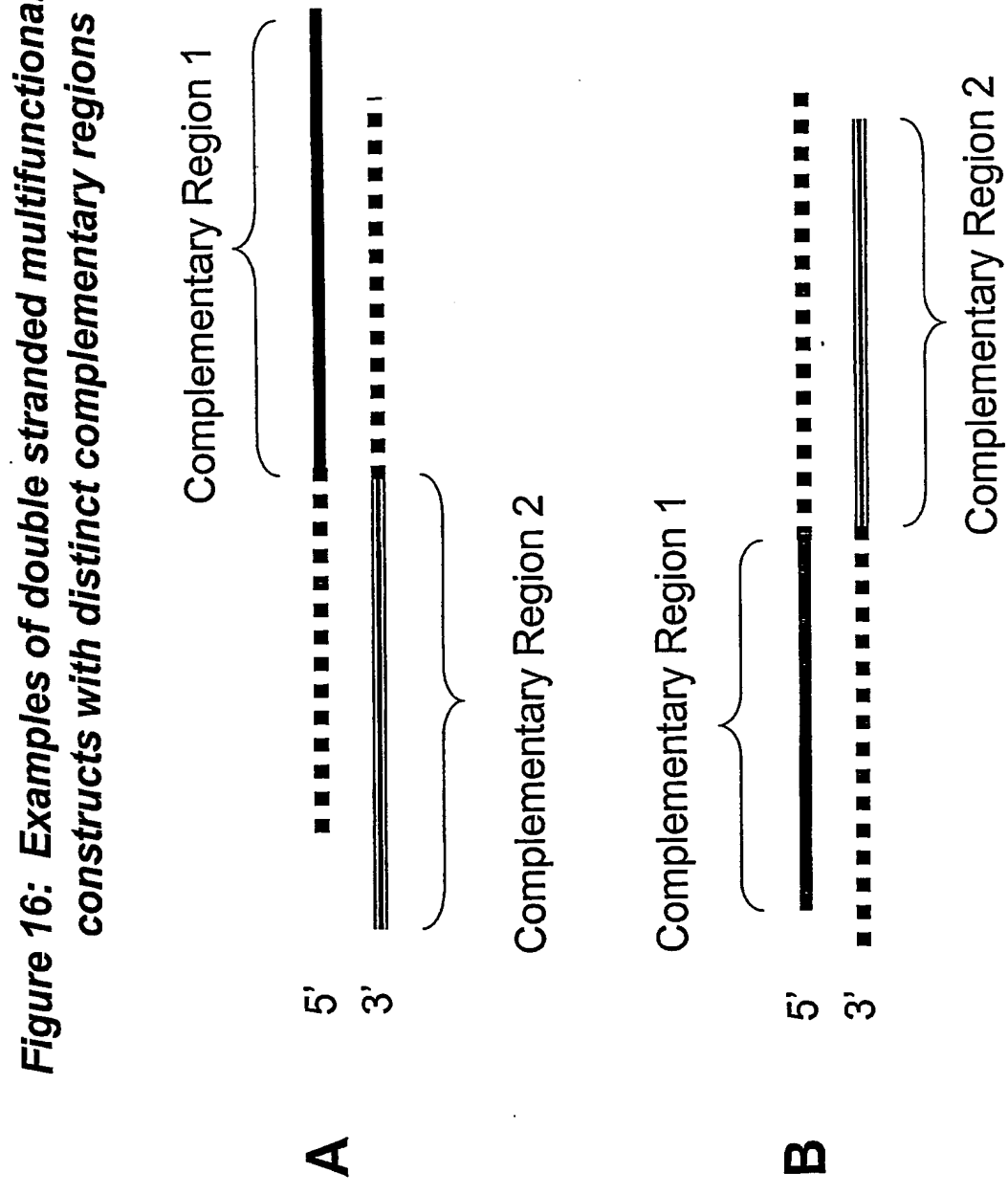


Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions



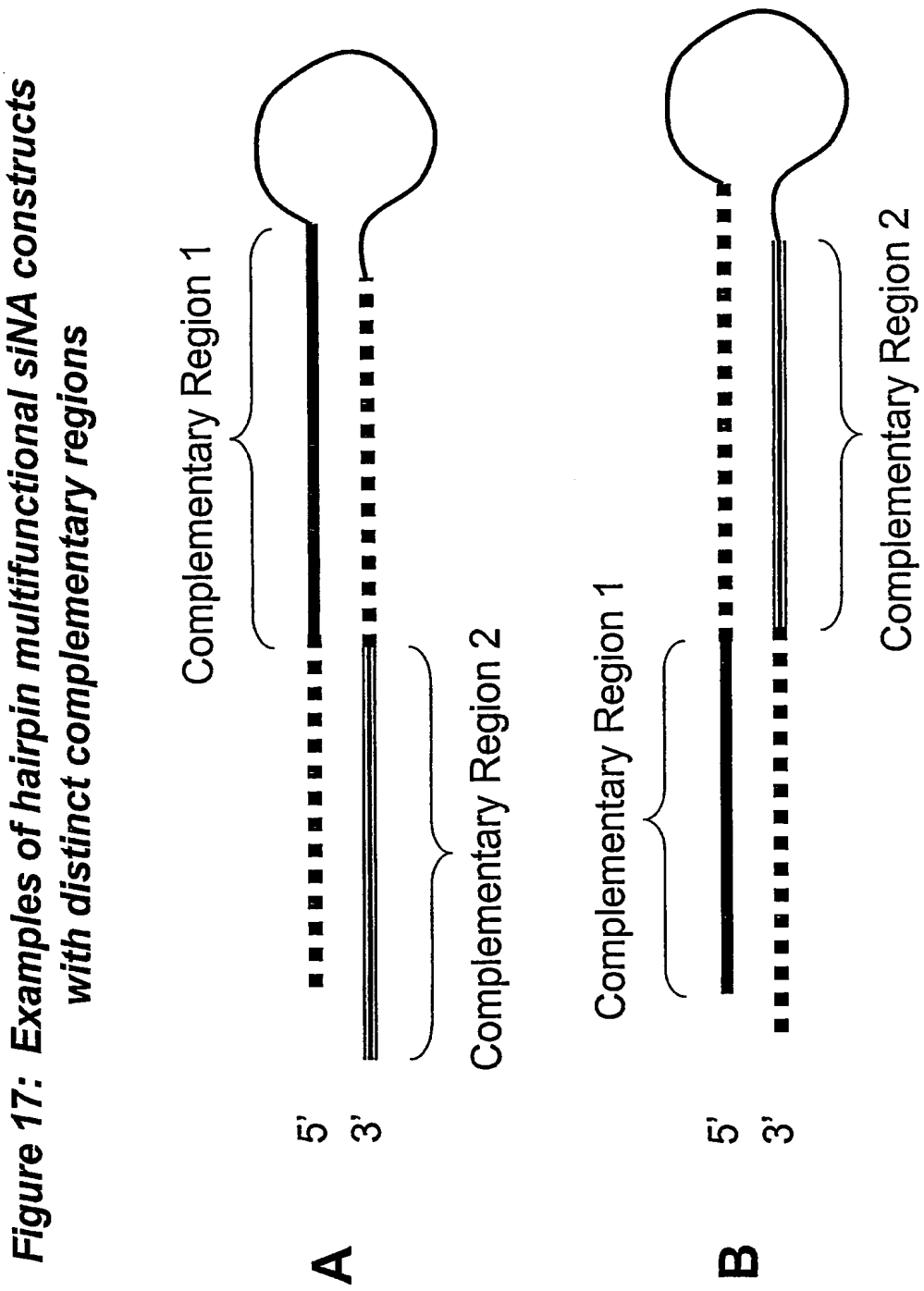
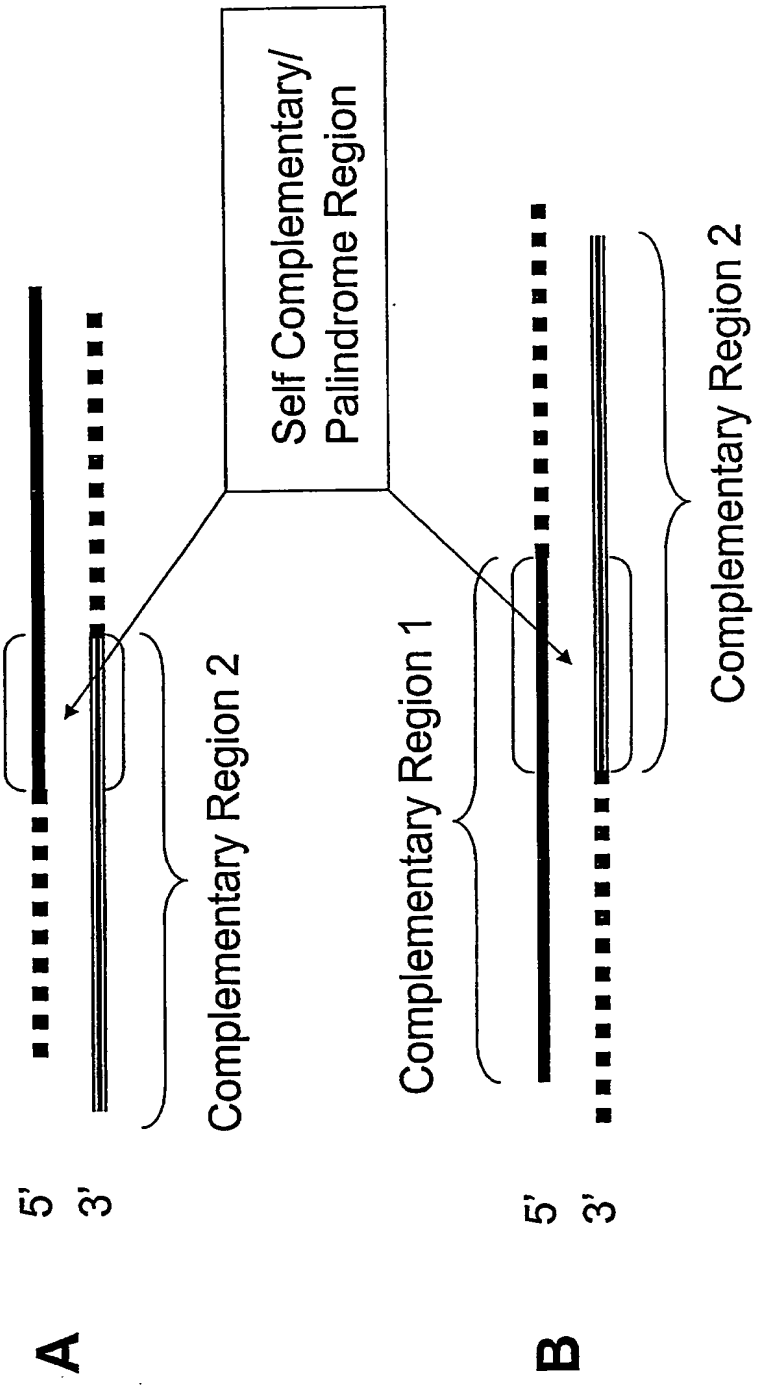


Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region



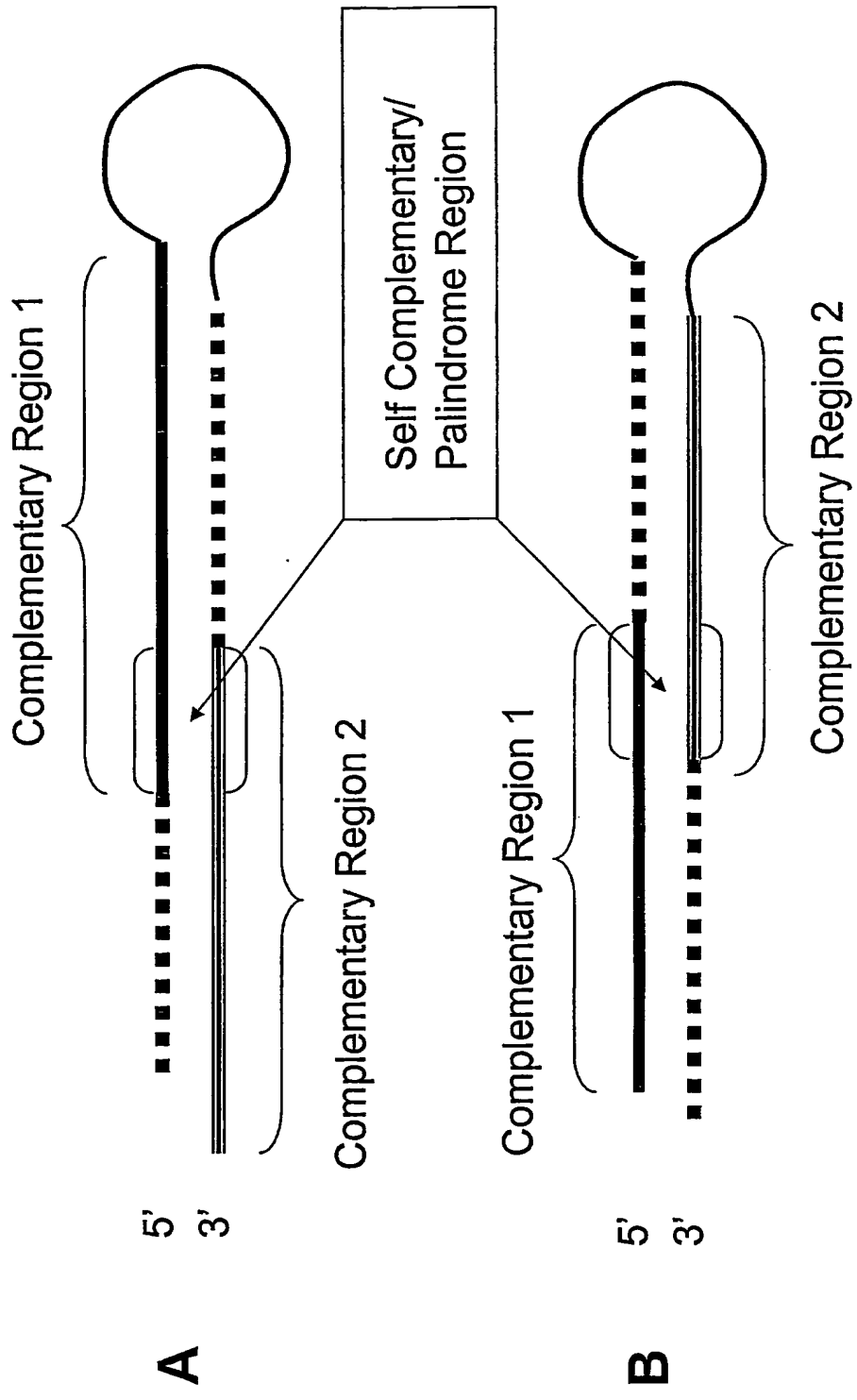


Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

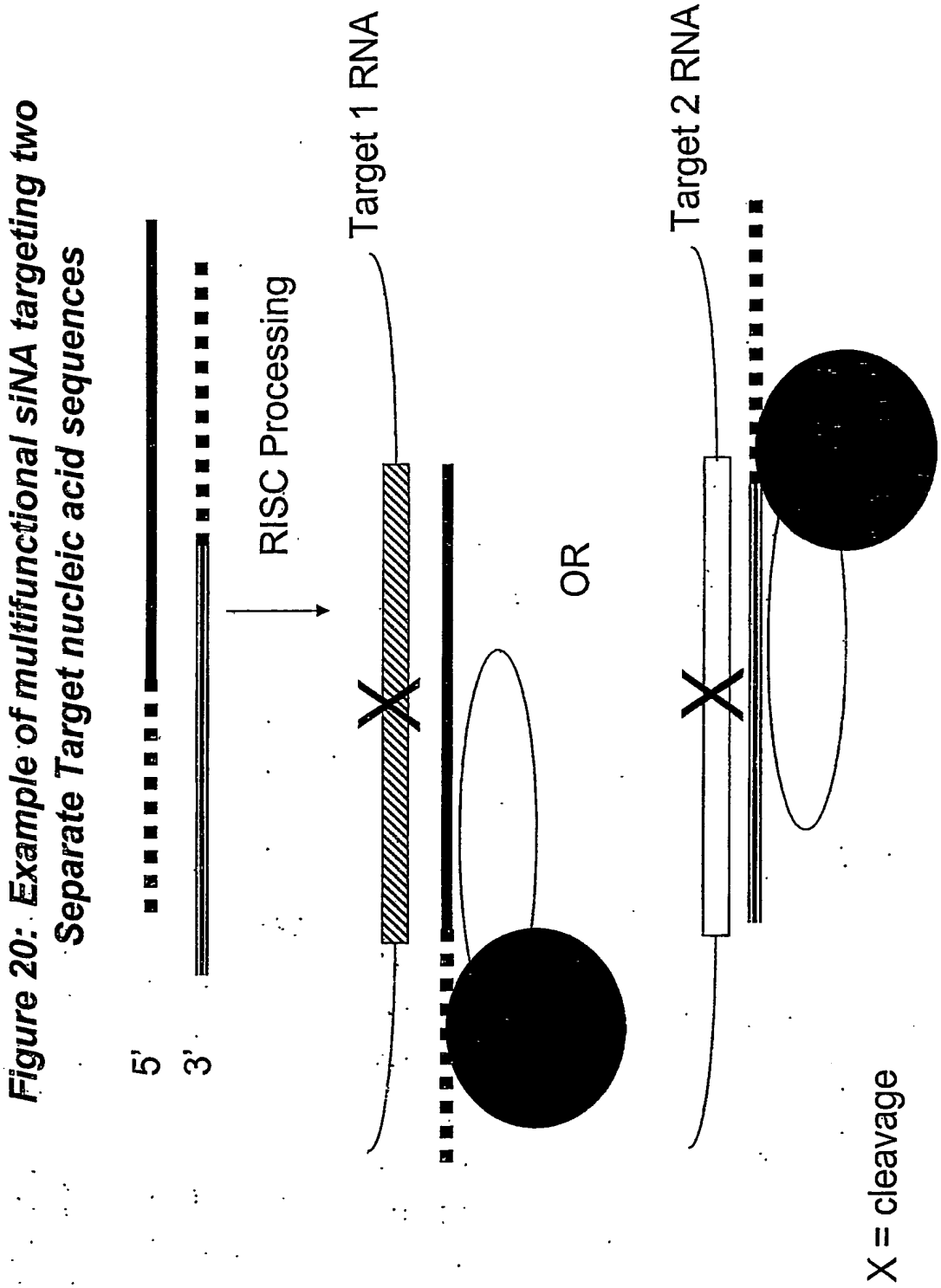


Figure 20: Example of multifunctional siNA targeting two Separate Target nucleic acid sequences

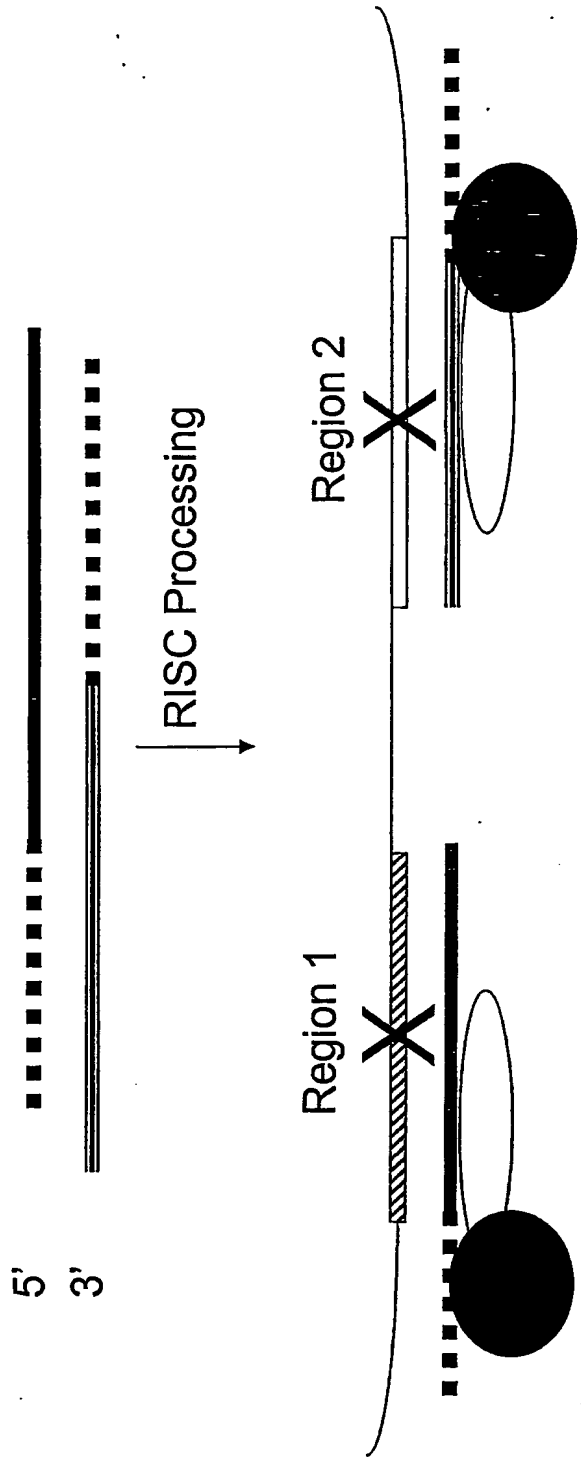
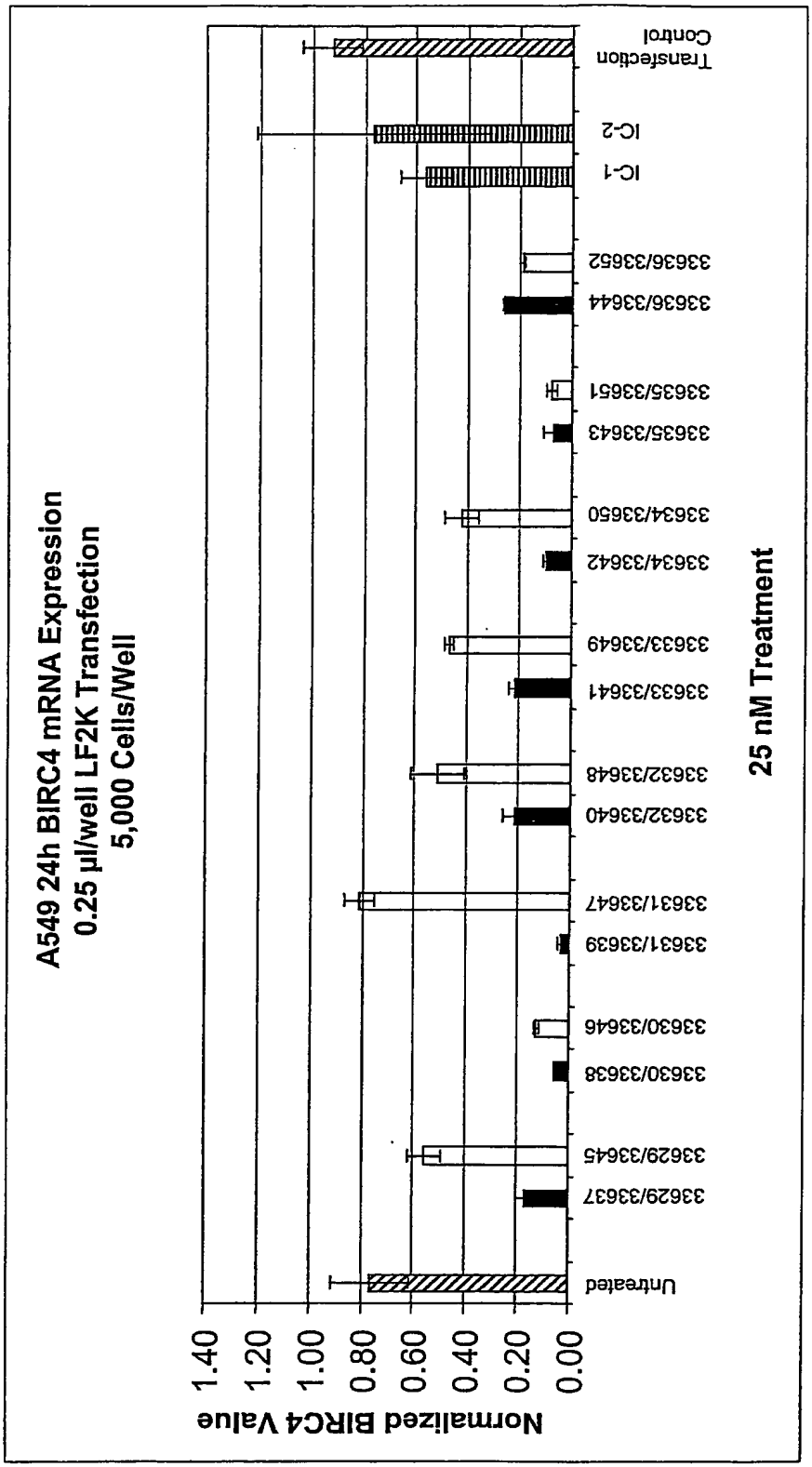


Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence

FIGURE 22


A549 24h BIRC4 mRNA Expression
0.25 µl/well LF2K Transfection
5,000 Cells/Well
25 nM Treatment
Normalized BIRC4 Value
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Untreated
33629/33637
33629/33645
33630/33638
33630/33646
33631/33639
33631/33647
33632/33640
33632/33648
33633/33641
33633/33649
33634/33642
33634/33650
33635/33643
33635/33651
33636/33644
33636/33652
IC-1
IC-2
Transfection Control